

# Protocol for expressing circularized protein in Origami cells

## Overnight cultures

**Material:**

- 20 ml LB medium
- 20 µl antibiotic (Chloramphenicol)
- 100 µl Origami from glycine stock

LB	1.0 % (w/v) Trypton	pH 7.0 with NaOH
	0.5 % (w/v)	
	Yeastextract	
	1.0 % (w/v) NaCl	

**Method:**

Add 5 µl antibiotic and one colony to 5 ml of LB medium and incubate at 37 °C overnight.

## Preparation of electrocompetent cells

**Material:**

- Overnight culture
- Ice
- Centrifuge
- 10 % Glycerin
- H<sub>2</sub>O

**Method:**

Take 1 ml or more from an overnight culture into 50 ml LB medium. Grow the cells until they reach an optical density at 600 nm of 0.5 – 0.6. Incubate the bacteria 20 min on ice. Centrifuge for 10 min at 4 °C and discard the supernatant. Add 20 ml cold H<sub>2</sub>O and resuspend the pellet. Centrifuge again for 10 min at 4 °C. Add 20 ml cold H<sub>2</sub>O and centrifuge for 10 min at 4 °C. Discard the supernatant and add 20 ml cold H<sub>2</sub>O. Centrifuge for 10 min at 4 °C. Discard the supernatant and add 10 ml cold glycerin (10 %). Resuspend the pellet and centrifuge at 4 °C for 10 min. discard the supernatant and resuspend the pellet in 2 ml 10 % cold glycerin. Take 100 µl aliquots into 1.5 µl reaction tubes. Froze them in liquid nitrogen and store at - 80 °C or use them directly for transformation.

## Transformation with electroporation

**Material:**

- 50 µl competent E.coli cells (Origami)
- 15 µl plasmid DNA
- Electroporator
- LB plates with chloramphenicol
- 200 µl LB

**Method:**

Add 50 µl competent cells and 1 µl plasmid DNA in a cuvette and use the electroporator for an electroshock. Add 200 µl LB in the cuvette and mix it. Put everything in a 1.5 ml reaction tube and incubate it for 30 minutes up to 1 hour at 37 °C. Plate on LB with chloramphenicol.

## Expression

**Material:**

- 2.5 ml from each overnight culture (Origami with iGEM\_02 Ax7L-DS and iGEM\_02 Ax7R-RR)
- 125 ml LB (for each culture)
- Antibiotic
- IPTG (2 mM)
- Centrifuge

**Method:**

Add 2.5 ml from each overnight culture to 125 ml LB. Measure the optical density at 600 nm. Let the bacteria grow until they reach an optical density about 0.5. Now add 2 mM IPTG to induce the expression of the TALE protein.

After 1 hour and 30 min, stop expression and centrifuge for 10 minutes. Store at -80 °C.

## Strep Tag Purification

**Material:**

- Pellet from Protein expression
- TE buffer
- PMSF
- Centrifuge
- Ultrasonic
- Twin Strep Tag Purification Kit from Iba (0.2 ml column)

**Method:**

Take a little bit from expression pellet and resuspend in 2 ml TE buffer and 20 µl PMSF. Use the ultrasonic program: 30 sec ultrasonic and 30 sec pause 8 times.

Centrifuge the Samples for 15 min at 4 °C and 20000 rpm

Now use the protocol from the Twin Strep Tag Purification Kit. First add 2 times of the column bed volume (CV, 0.2 ml) of Buffer W. Apply 0.1 up to 10 of the CV from the supernatant (from the centrifuged Samples) to the column.

When the liquid completely entered the column, add 1 CV washing buffer w. Repeat this step 4 times.

After finishing the washing step, add 0.5 CV washing buffer W. Repeat it 5 times until there are 6 elution Samples.

Regenerate the column three times with 5 CV regeneration buffer R.

Finish the regeneration by adding 2 times 4 CV washing buffer w and store the column at 4 °C with 2 ml washing buffer w.

## TCA precipitation

### **Material:**

Samples (from strep tag purification)

NaDOC

Ice

TCA

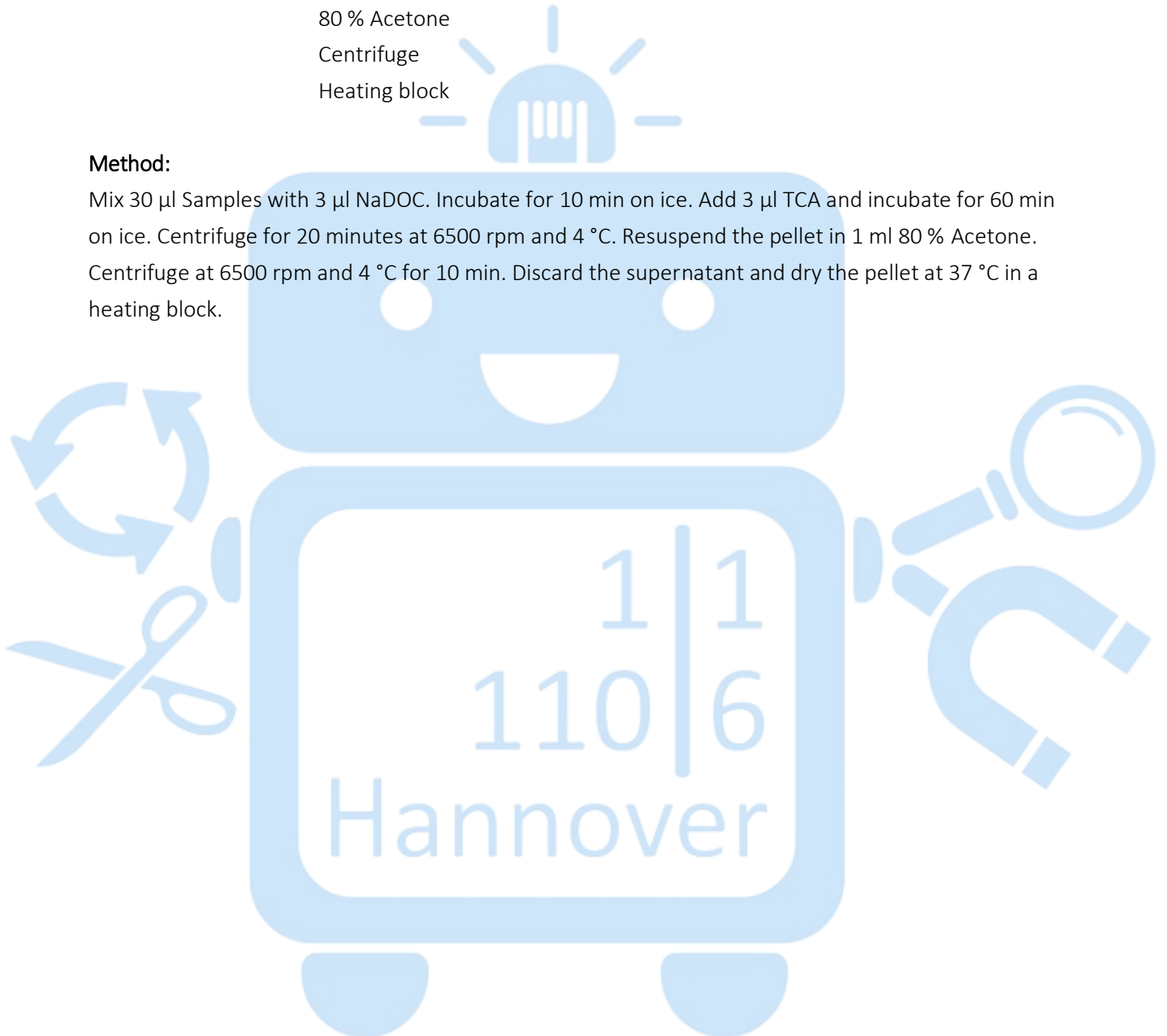
80 % Acetone

Centrifuge

Heating block

### **Method:**

Mix 30 µl Samples with 3 µl NaDOC. Incubate for 10 min on ice. Add 3 µl TCA and incubate for 60 min on ice. Centrifuge for 20 minutes at 6500 rpm and 4 °C. Resuspend the pellet in 1 ml 80 % Acetone. Centrifuge at 6500 rpm and 4 °C for 10 min. Discard the supernatant and dry the pellet at 37 °C in a heating block.



## SDS-PAGE

### **Material:**

For 10 Gels:

**Separating gel (10 %):** 27.25 ml H<sub>2</sub>O  
33.25 Acrylamid (30 %)  
25 ml Tris (1.5 M; pH 8.8)  
1 ml APS (10 %)  
1 ml SDS (10 %)  
40 µl TEMED

**Stacking gel:** 34 ml H<sub>2</sub>O  
8.5 ml Acrylamid (30 %)  
6.25 ml Tris (0.5 M; pH 6.8)  
0.5 ml APS (10 %)  
0.5 ml SDS (10 %)  
50 µl TEMED

**TANK buffer:** 30 g Tris  
144 g Glycine  
100 ml 10 % SDS

### **Samples:**

20 µl with column purified Sample (or a little bit of the Expression pellet)  
20 µl Lämmli (protein loading buffer)  
7 – 10 µl Page Ruler Prestained protein ladder

### **Method:**

For preparing an SDS Gel start with the separating gel. Bring everything together in this order:

1. H<sub>2</sub>O, 2. Tris, 3. SDS, 4. Acrylamide, 5. APS, 6. TEMED; APS and TEMED every time at last! After adding them be fast. Pour it into the gel apparatus and cover it with 1 ml Isopropanol. Wait until it got solid. Then prepare the stacking gel. Get everything in the same order together (1. H<sub>2</sub>O, 2. Tris, 3. SDS, 4. Acrylamide, 5. APS, 6. TEMED). Remove the Isopropanol and pour the stacking gel above the separating gel.

Prepare the Samples. Take 20 µl of each sample and add 20 µl loading buffer. Incubate for 10 min at 100 °C. Load 20 µl of each prepared Sample into the gel and run the gel at 150 V for 40 min or until the first band of the protein ladder reached the end of the gel.

## Semi dry Western blot

### **Material:**

Blotting chamber  
Power supply  
PVDF membrane  
6 filter paper  
SDS Gel

### ***Toubin:***

5.81 g Tris  
400 µl 10 % SDS  
2.89 g Glycine  
200 ml Methanol  
Add 1 l H<sub>2</sub>O

### ***Ponceau solution:***

0.2 % Ponceau  
3 % TCA  
Store at room temperature

### ***Blocking solution:***

1 x RotiBlock  
1 x PBST

### **Method:**

Put the filter paper into the buffer, stack 3 filter papers, put the buffer soaked membrane on the filter paper, lay the gel on the membrane and stack again 3 filter papers soaked with buffer at the gel.

Remove the air in the stack and run the Transfer at 5.5 V per 1 cm<sup>2</sup> for 40 min.

Incubate the membrane for 10 min in ponceau solution. Destain the membrane with H<sub>2</sub>O and incubate the membrane at least for one hour in blocking solution.

## Coomassie staining

### **Material:**

***Coomassie staining solution:*** 0.05 % Coomassie R250  
25 % Isopropanol  
10 % glacial acetic acid

***Destaining solution:*** 10 % glacial acetic acid or H<sub>2</sub>O

### **Method:**

Add Coomassie to the gel at least for 20 min. Remove the coomassie staining solution and add the destaining solution or water until the redundant coomassie is gone.

## Immunostain

### Material:

Pincette

Bowl for Immunostain

Rocker

#### **10 x PBS:**

2.56 g  $\text{NaH}_2\text{PO}_4 \times \text{H}_2\text{O}$

14.90 g  $\text{Na}_2\text{HPO}_4$

87.66 g NaCl

Ad 1 l  $\text{H}_2\text{O}$  pH 7.4

#### **PBST:**

PBS pH 7.4

0.05 % Tween20

#### **Blotto:**

1 x RotiBlock

1 x PBST

#### **Substrate solution per membrane:**

6 ml 100 mM Tris pH 8.5

30  $\mu\text{l}$  Luminol (22.2 mg/ 500  $\mu\text{l}$  DMSO)

15  $\mu\text{l}$  p-Cunaracid

(7.4 mg/ 500  $\mu\text{l}$  DMSO)

10  $\mu\text{l}$  30 %  $\text{H}_2\text{O}_2$

### Antibody 1:

StrepMAB – Classic cell culture supernatant IgG1 Anti – Strep – tagII monoclonal  
2-1508-025 Iba Life Sciences

### Antibody 2:

ECL – Anti-mouse IgG Horseradish Peroxidase-linked whole antibody  
GE healthcare UK NA931V lot 9682503

### Method:

Wash the membrane with PBST. Incubate the membrane with the first antibody (1:2000 in blotto) for one hour while shaking.

Wash the membrane 1 x short with PBST, 1 x 5 min with PBST.

Add the second antibody (1:10000 in blotto) and incubate for one hour while shaking.

Wash the membrane 1 x short with PBST, 1 x 5 min with PBST.

Incubate with substrate buffer for a short time (30 sec up to 20 min).

### TEV digest

**Material:**

- TEV Protease
- TEV Buffer
- DTT
- Purified protein
- H<sub>2</sub>O

**Method:**

Use 2 µl TEV Protease, 5 µl TEV buffer, 1 µl DTT, 40 µl purified Protein and 52 µl H<sub>2</sub>O. Incubate for 0 h, 1 h, 2 h, 4h and 6 h at room temperature. Take 20 µl Sample each time.

### Stability test

**Material:**

- purified protein
- Fridge
- Chamber with 37 °C

**Method:**

To test the stability of the circularized protein, different conditions were tested.

The protein was placed at room temperature, 37 °C and 4 °C. Samples were taken at 0 hours, 6 hours, 24 hours and 48 hours.

The other Samples were frost and defrost several times. Samples were taken at 0 times frost and defrost, 1-time frost and defrost, 2 times frost and defrost and 3 times frost and defrost.